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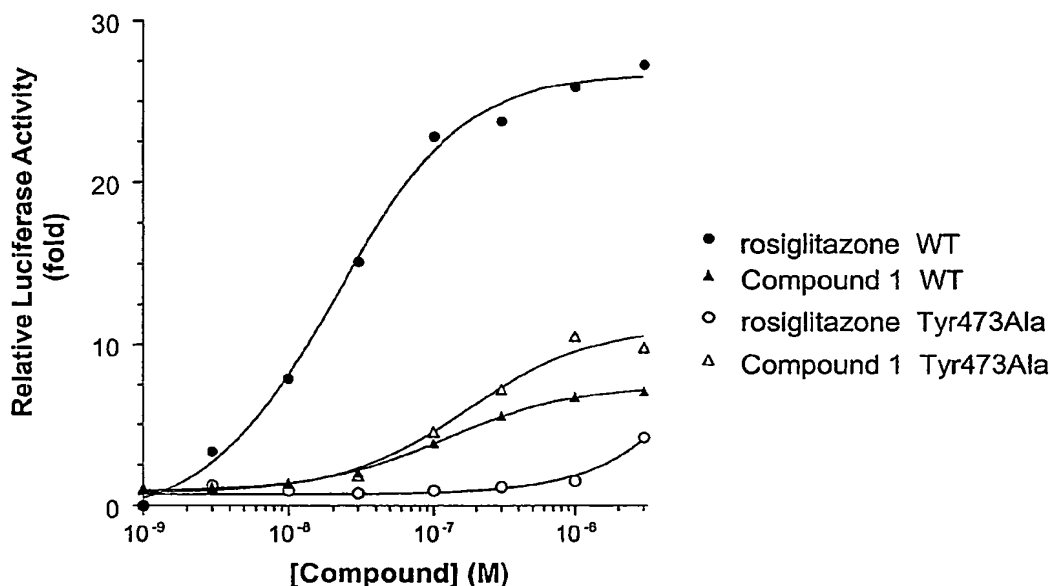
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(54) Title: PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR



(57) **Abstract:** The present invention features mutated forms of PPAR ligand binding domain polypeptides that: (1) bind a partial PPAR agonist; and (2) is bound or activated by a full PPAR agonist to a lesser extent than the wild-type receptor. The mutated ligand binding domain contains an amino acid sequence wherein one or more interactions that preferentially (preferably solely) occurs between a full PPAR agonist and the AF-2 domain of a wild-type PPAR are modified. Preferably, the mutated ligand binding domain is selectively bound or activated by a partial PPAR agonist.

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TITLE OF THE INVENTION

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

5 The present application claims the benefit of U.S. Provisional Application No. 60/441,836, filed January 22, 2003, hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 The references cited throughout the present application are not admitted to be prior art to the claimed invention.

 Nuclear receptors act as ligand-inducible transcription factors that regulate target gene expression. Regulation of target gene expression is mediated by complexes involving the nuclear receptor, agonist or antagonist ligands, and one or more coregulators. Depending on the nuclear receptor, the receptor may be present in the complex as a monomer, homodimer, or
15 heterodimer. (Aranda *et al.*, *Physiological Reviews* 81:1269-1304, 2001.)

 Different nuclear receptors respond to different ligands and regulate different genes. Examples of nuclear receptors include thyroid hormone receptor, retinoic acid receptor, vitamin D receptor, peroxisome proliferator-activated receptors, pregnane X receptor, constitutive androstane receptor, liver X receptor, farnesoid X receptor, reverse ErbA, retinoid Z
20 receptor/retinoic acid-related orphan receptor, ubiquitous receptor, retinoid X receptor, chicken ovalbumin upstream promoter transcription factor, hepatocyte nuclear factor 4, tailles-related receptor, photoreceptor-specific nuclear receptor, testis receptor, glucocorticoid receptor, androgen receptor, progesterone receptor, estrogen receptor, estrogen-related receptor, NGF-induced clone B, steroidogenic factor 1, fushi tarazu factor 1, germ cell nuclear factor, and
25 dosage-sensitive sex reversal. (Aranda *et al.*, *Physiological Reviews* 81:1269-1304, 2001.)

 Nuclear receptors exhibit a modular structure with different regions corresponding to autonomous functional domains that can be interchanged between related receptors. (Aranda *et al.*, *Physiological Reviews* 81:1269-1304, 2001.) A typical nuclear receptor comprises the following regions: (A/B) a variable amino terminal region containing the ligand independent
30 AF-1 domain; (C) a conserved DNA binding domain; (D) a variable linker region; and (E) a ligand binding domain region containing the ligand-dependent AF-2 core transactivation domain. (Aranda *et al.*, *Physiological Reviews* 81:1269-1304, 2001.)

 An important subfamily of nuclear receptors are peroxisome proliferator activated receptors (PPAR's). The PPAR subfamily of nuclear receptors includes PPAR α , PPAR γ , and
35 PPAR δ (also known as PPAR β), and these receptors function as heterodimers with the retinoid

X receptor (RXR). Fatty acids and eicosanoids have been identified as naturally occurring PPAR ligands. (Berger *et al.*, *Annu. Rev. Med.* 53:409-435, 2002, Berger *et al.*, *Diabetes Technology & Therapeutics* 4:163-174, 2002.)

5 Agonist or partial-agonist binding to a PPAR induces stabilization of the structure as well as a change in conformation that creates a binding cleft resulting in recruitment of transcriptional coactivators. Examples of PPAR coactivators include CBP/p300, the steroid receptor coactivator (SRC-1), members of the DRIP/TRAP complex, PGC-1, RIP140, and ARA70. The active PPAR complex is bound to a specific DNA response element mediating the rate of initiation of gene transcription. (Berger *et al.*, *Annu. Rev. Med.* 53:409-435, 2002, Berger
10 *et al.*, *Diabetes Technology & Therapeutics* 4:163-174, 2002.)

Different synthetic compounds modulating a PPAR activity have been identified. (See, e.g., Berger *et al.*, *Annu. Rev. Med.* 53:409-435, 2002, Berger *et al.*, *Diabetes Technology & Therapeutics* 4:163-174, 2002, Acton *et al.* International Publication Number WO 02/08188, published January 31, 2002, Berger *et al.*, International Publication Number WO 01/30343,
15 published May 3, 2001, Cobb *et al.*, International Publication Number WO 01/17944, published March 15, 2001.)

Partial agonists (or antagonists), also known as "selective modulators" for PPAR's have been strongly implicated as having preferred biological properties (Berger *et al.*, International Publication Number WO 01/30343, published May 3, 2001, Moller, *Nature*
20 414:821-827, 2001, Berger *et al.*, *Annu. Rev. Med.* 53:409-435, 2002). These may include the retention of selected responses which confer efficacy whereas selected responses that result in toxicity may be diminished.

SUMMARY OF THE INVENTION

25 The present invention features mutated forms of PPAR ligand binding domain polypeptides that: (1) bind a partial PPAR agonist; and (2) is bound or activated by a full PPAR agonist to a lesser extent than the wild-type receptor. The mutated ligand binding domain contains an amino acid sequence wherein one or more interactions that preferentially (preferably solely) occurs between a full PPAR agonist and the AF-2 domain of a wild-type PPAR are
30 modified. Preferably, the mutated ligand binding domain is selectively bound or activated by a partial PPAR agonist.

Selective binding or activation by a partial PPAR agonist is in comparison to activation by a full PPAR agonist. A full PPAR agonist is either a potent natural ligand or has the same type of interactions with PPAR AF-2 domain amino acids as a potent natural ligand. In

contrast, a partial agonist has a significantly diminished interaction with one or more amino acids that are important for full agonist binding or activation.

A “partial PPAR agonist” can bind to a wild-type PPAR and cause detectable receptor activity, where the produced activity is less than the activity caused by a full ligand. Differences between partial and full agonist produced activity can be the type or degree of activity.

Depending upon the extent of activation caused by a partial PPAR agonist, the partial agonist can be used as an agonist or an antagonist. A partial agonist can be used in an antagonist manner, for example, by competing and diluting the effect of a naturally occurring agonist.

The ability of a mutated PPAR ligand binding domain to selectively bind a partial agonist indicates: (1) a partial agonist can bind to the mutated ligand binding domain at a comparable or greater level than it binds to the wild-type protein; and (2) a full agonist binds to the mutated ligand binding domain to a lesser extent than to the wild-type protein at a given concentration, or binds to the wild-type protein to a comparable extent, but only at a higher concentration.

The ability of a mutated PPAR ligand binding domain to be selectively activated by a partial agonist indicates: (1) a partial agonist can produce a comparable or greater response in a PPAR containing the mutated ligand binding domain than in the wild-type protein; and (2) a full agonist produces a lesser response in a PPAR containing the mutated ligand binding domain than in the wild-type protein at a given concentration, or produces a response comparable to that in the wild-type protein, but only at a higher concentration.

Reference to a “mutated” PPAR ligand binding domain indicates a different amino acid sequence than a wild-type PPAR ligand domain. Reference to “mutated” does not indicate the manner in which the “mutated” domain was produced. A “mutated” PPAR ligand binding domain can be obtained by different methods including those involving introducing a mutation into a PPAR ligand binding domain encoding nucleotide sequence, step-wise chemical synthesis of a PPAR encoding nucleotide sequence to express a “mutated” ligand binding domain, and chemically synthesizing a particular PPAR ligand binding domain amino acid sequence.

Thus, a first aspect of the present invention features a mutated PPAR ligand binding domain polypeptide. The polypeptide comprises the amino acid sequence of a mutated PPAR ligand binding domain, wherein the mutated PPAR ligand binding domain is:

(a) bound by a partial PPAR agonist; and

(b) bound or activated by a full PPAR agonist to a lesser extent than the wild-type receptor.

Activation of a mutated PPAR ligand binding domain polypeptide can be, for example, a change in conformation that would allow recruitment or binding of coactivator proteins.

Unless particular terms are mutually exclusive, reference to “or” indicates either or both possibilities. Thus, for example, reference to “bound or activated” includes bound, activated and both bound and activated.

Another aspect of the present invention describes a mutated PPAR ligand binding domain polypeptide that is a ligand-activated transcription factor. The ligand-activated transcription factor comprises a mutated PPAR ligand binding domain and a transcription factor DNA binding domain. The ligand-activated transcription factor is bound to the DNA response element targeted by the DNA binding domain.

A ligand-activated transcription factor may contain a mutated PPAR ligand binding domain from a particular PPAR subtype along with other PPAR regions from that subtype or may be a chimeric ligand-activated transcription factor. A chimeric ligand-activated transcription factor described herein contains a mutated PPAR ligand binding domain from a particular subtype along with one or more regions from a different nuclear receptor.

Another aspect of the present invention describes a method of making a mutated PPAR ligand binding domain polypeptide. The method involves mutating a PPAR ligand binding domain such that an amino acid present in a wild-type PPAR ligand binding domain that makes a direct interaction with a full agonist is replaced with an amino acid that either makes no interaction, or a substantially different interaction, with the full agonist. If desired additional alterations can be made.

Another aspect of the present invention describes a nucleic acid comprising a nucleotide sequence encoding a mutated PPAR ligand binding domain polypeptide.

Another aspect of the present invention describes a recombinant cell comprising nucleic acid containing a nucleotide sequence encoding a mutated PPAR ligand binding domain polypeptide, wherein the nucleic acid is expressed in the cell. Reference to “expressed” indicates the production of encoded polypeptide.

Another aspect of the present invention describes a method of assaying for a partial PPAR agonist. The method involves measuring the ability of a test compound to bind or activate a mutated PPAR ligand binding domain polypeptide or a transcription factor containing a mutated PPAR ligand binding domain. Measuring can be performed qualitatively or quantitatively.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodologies useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodologies useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides the amino acid sequence of a wild type PPAR α (SEQ ID NO: 1). Tyr464 is shown in bold. The ligand binding domain is from about amino acid 281 to 468. The DNA binding domain is from about amino acids 102 to 166.

Figure 2 provides the amino acid sequence of a wild type PPAR δ (SEQ ID NO: 2). Tyr437 is shown in bold. The ligand binding domain is from about amino acid 254 to 441. The DNA binding domain is from about amino acids 74 to 138.

Figure 3 provides the amino acid sequence of a wild type PPAR γ (SEQ ID NO: 3). Tyr473 is shown in bold. The ligand binding domain is from about amino acid 203 to 477. The DNA binding domain is from about amino acids 81 to 145.

Figure 4 illustrates Compound 1 and rosiglitazone-induced transactivation of a PPAR γ Tyr473Ala mutant in comparison with wild-type PPAR γ response.

Figure 5 illustrates Compound 1 and rosiglitazone-induced transactivation of a PPAR γ Tyr473Phe mutant in comparison with wild-type PPAR γ response.

DETAILED DESCRIPTION OF THE INVENTION

Polypeptides containing mutated PPAR ligand binding domains described herein can be used to facilitate identification and evaluation of partial agonists. Partial agonists have research and therapeutic applications. Research applications include using the partial agonist to study the biological effects of PPAR partial activation or antagonism and to identify important functional groups affecting the ability of a partial agonist to bind to or modulate a PPAR activity.

Therapeutic applications include using those partial agonists having appropriate pharmacological properties such as efficacy and lack of unacceptable toxicity to achieve a beneficial effect in a patient. A partial agonist can be used to provide a beneficial effect of PPAR modulation (e.g., partial activation or antagonism), while producing less side effects than a full agonist.

A "patient" refers to a mammal that can receive a beneficial effect by the administration of a PPAR partial agonist. A patient can be treated prophylactically or

therapeutically. Examples of patients include human patients, and non-human patients such as farm animal, pets, and animals that can be used as model systems.

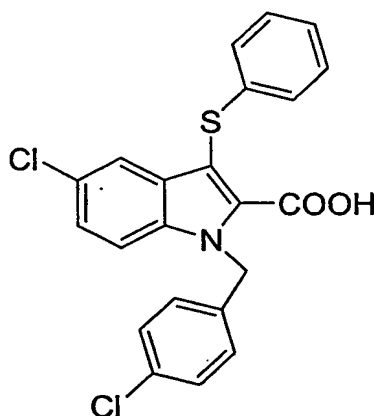
Beneficial effects that can be achieved by modulating one or more PPARs include treatment of one or more of the following: atherosclerosis, dyslipidemia, inflammation, cancer, infertility, hypertension, obesity, and diabetes. (Berger *et al.*, *Annu. Rev. Med.* 53:409-435, 2002, Berger *et al.*, *Diabetes Technology & Therapeutics* 4:163-174, 2002, Berger *et al.*, International Publication Number WO 01/30343, published May 3, 2001.)

PPAR γ

Using the PPAR γ ligand binding domain as a model it was found that alterations can be produced resulting in a mutated ligand binding domain that is selectively bound or activated by a partial agonist. The mutated ligand binding domains illustrated in the Examples *infra* have a Tyr473Ala or Tyr473Phe substitution.

The full agonist rosiglitazone hydrogen bonds with the PPAR γ Tyr473 phenolic hydroxyl, while the partial agonist 1-(p-chlorobenzyl)-5-chloro-3-phenylthiobenzyl-2-yl carboxylic acid (Compound 1) does not hydrogen bond with Tyr473. Replacement of Try473 with an amino acid that does not allow hydrogen bonding to rosiglitazone diminishes an interaction that occurs between rosiglitazone and the AF-2 domain.

Compound 1 and its use as a partial agonist is described by Berger *et al.*, International publication WO 01/30343, published May 3, 2001. Compound 1 has the following structure:



PPAR γ ligand binding domain polypeptides in which Tyr473 was replaced with a non-polar amino acid (*e.g.*, alanine or phenylalanine) were found to bind to partial agonist and to activate ligand binding domain activity. Activation of a transcription factor containing a

mutated ligand binding domain was at least as good (Tyr473Ala) or significantly better (Tyr473Phe) than that occurring with the wild-type ligand binding domain.

Amino acids involved in agonist and partial agonist binding can be identified using X-ray crystallography. PPAR γ ligand binding domain X-ray crystallography data, and techniques for generating such data are illustrated by, for example, Nolte *et al.*, *Nature* 395:137-143, 1998 and Oberfield *et al.*, *Proc. Natl. Acad. Sci. USA* 96:6120-6106, 1999.

Amino acids other than Tyr473 can be mutated to diminish binding of a full agonist to the PPAR γ AF-2 domain and maintain or facilitate partial agonist binding or activity. The ability of a polypeptide containing a mutated ligand binding domain to be selectively activated or bound by a partial agonist can be evaluated by, for example, measuring the ability of the polypeptide to bind or be activated by a full agonist and partial agonist.

Reference to an amino acid in a particular location such as Tyr473 is with respect to a reference amino acid sequence. Reference amino acid sequences for PPAR α , PPAR δ , PPAR γ are provided by SEQ ID NOs: 1, 2 and 3 (Figure 1-3). The amino acid numbering for a particular PPAR may differ due to differences in that PPAR that occur in nature or are artificially produced. Naturally occurring differences may be, for example, isoforms and polymorphisms.

The amino acid in a polypeptide corresponding to a referenced amino acid can readily be identified by performing a sequence alignment with a reference sequence. The alignment should be performed to maximize the number of identical amino acids in a region (*e.g.*, 15 or 20 amino acids) containing the amino acid in question.

Replacement of tyrosine 473 with an appropriate amino acid could produce a mutated human PPAR γ ligand binding domain with unique properties that can be used to identify the kinds of ligands used to activate the nuclear receptor. In different embodiments, the ligand binding domain is a mutated human PPAR γ ligand binding domain, wherein a residue corresponding to tyrosine 473 is selected from a group consisting of:

- (a) alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine;
- (b) alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine; or
- (c) alanine or phenylalanine.

In another embodiment, the ligand binding domain comprises SEQ ID NO: 4 or a structurally similar sequence. SEQ ID NO: 4 is provided as follows:

QLNPESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVTYDMNSLMMGEDKI
KFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLKYGVBH
EIIYTMLASLMNKDGVLISEGQGFMTRFLKSLRKPFPGDFMEPKFEFAVKFNALELDDSD

LAIFIAVILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESQLFAKLLQKMTDLRQI
VTEHVQLLQVIKKTETDMSLHPLLQEIXKDLY

wherein X is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine. In further
5 embodiments X is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine; and X is alanine or phenylalanine.

PPAR α and PPAR δ

PPAR α , PPAR δ , and PPAR γ contain similar ligand binding domains, where the
10 AF-2 domain contributes to the ligand binding pocket. The AF-2 domain in these receptors provides a ligand-dependent activation domain that participates in the generation of a coactivator binding pocket. (Berger *et al.*, *Annu. Rev. Med.* 53:409-435, 2002.)

The similarity between different PPAR ligand binding domains and the results
obtained using a mutated PPAR γ ligand binding domain can be used to guide the design of
15 polypeptides containing a mutated PPAR α or PPAR δ ligand binding domain. The ability of a polypeptide containing a mutated ligand binding domain to be selectively activated or bound by a partial agonist can be evaluated by, for example, measuring the ability of the polypeptide to bind or be activated by a full agonist and partial agonist.

X-ray crystallography data for PPAR α and PPAR δ can be generated using
20 techniques well known in the art. X-ray crystallography data for the PPAR α ligand binding domain and ligand binding is described by Lambert *et al.*, International Publication Number WO 02/064632, published August 22, 2002. X-ray crystallography data for the PPAR δ ligand binding domain and ligand binding is described by Xu *et al.*, *Molecular Cell* 3:397-403, 1999.

PPAR α and PPAR δ contain tyrosine residues that function in an analogous
25 manner to Tyr473 in PPAR γ . The analogous PPAR α tyrosine is in position 464 (Figure 1). The analogous PPAR δ tyrosine is in position 437 (Figure 2).

Partial agonists for PPAR α can be identified, for example, by screening for
compounds that activate PPAR α where Tyr464 is replaced with an amino acid such as alanine or phenylalanine. Such partial agonists, in addition to the other uses described herein, can be used
30 to obtain or evaluate mutated PPAR α ligand binding domain polypeptides and ligand-activated transcription factors.

Similarly, partial agonists for PPAR δ can be identified, for example, by screening
for compounds that activate PPAR δ where Tyr437 is replaced with an amino acid such as alanine or phenylalanine. Such partial agonists, in addition to the other uses described herein,

can be used to obtain or evaluate mutated PPAR δ ligand binding domain polypeptides and ligand-activated transcription factors.

A mutated human PPAR α ligand binding where tyrosine 464 is replaced with an appropriate amino acid could produce a mutated human PPAR α ligand binding domain with unique properties that can be used to identify the kinds of ligands used to activate the nuclear receptor. Similarly, a mutated human PPAR δ ligand binding where tyrosine 437 is replaced with an appropriate amino acid could produce a mutated human PPAR δ ligand binding domain with unique properties that can be used to identify the kinds of ligands used to activate the nuclear receptor.

In different embodiments, the mutated ligand binding domain either is a mutated human PPAR α ligand binding domain containing a mutation in a residue corresponding to tyrosine 464, or a mutated human PPAR δ ligand binding domain containing a mutation in a residue corresponding to tyrosine 437, wherein the mutation is an amino acid selected from the group consisting of: (a) alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine. In further embodiments, the mutation is either an amino acid selected from the group consisting of alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine; or is alanine or phenylalanine.

Ligand-Activated Transcription Factor

A ligand-activated transcription factor binds a partial agonist and can modulate gene expression upon partial agonist binding. Based on the interchangeability of different nuclear receptor regions, different types of transcription factors can be produced containing a mutated PPAR ligand binding domain.

Nuclear receptors exhibit a modular structure with different regions corresponding to autonomous functional domains that can be interchanged between related receptors. (Aranda *et al.*, *Physiological Reviews* 81:1269-1304, 2001.) In different embodiments, a ligand-activated transcription factor is a chimeric receptor containing a mutated PPAR ligand binding domain and one or more regions from another nuclear receptor or other transcription factor (such as GAL4); or is a particular PPAR having a mutated ligand binding domain.

A preferred chimeric receptor described herein is one containing a mutated PPAR ligand binding domain and a DNA binding domain from a different nuclear receptor or other transcription factor (such as GAL4). The selection of a particular DNA binding domain is useful in designing a reporter system to measure receptor activity. Examples of DNA binding domains used in PPAR chimeric receptors are the yeast transcription factor Gal4 and the glucocorticoid receptor. (Lehman *et al.*, *The Journal of Biological Chemistry* 270:12953-12956, 1995, Schmidt

et al., *Molecular and Cellular Endocrinology* 155:51-60, 1999, Berger *et al.*, *The Journal of Biological Chemistry* 274:6718-6725, 1999.)

Ligand binding domain regions based on a PPAR can be designed starting from known PPAR sequences. Different PPAR α , PPAR δ , PPAR γ sequences include different isoforms and polymorphisms. References providing PPAR α sequence information include Sher *et al.*, *Biochemistry* 32:5598-5604, 1993 (see also SWISS-PROT: QO7869). References providing PPAR γ sequence information include Elbrecht *et al.*, *Biochem. Biophys. Res. Commun.* 224:431-437, 1996 (see also SWISS-PROT: P37231). References providing PPAR δ sequence information include Schmidt *et al.*, *Mol. Endocrinol.* 6:1634-1641, 1993, (see also SWISS-PROT: QO3181).

X-ray crystallography data pointing out the importance of different PPAR amino acid residues to ligand binding and activity can be used to facilitate polypeptide design. References providing examples of X-ray crystallography data and methods of obtaining such data include Lambert *et al.*, International Publication Number WO 02/064632, published August 22, 2002, Xu *et al.*, *Molecular Cell* 3:397-403, 1999, Nolte *et al.*, *Nature* 395:137-143, 1998, and Oberfield *et al.*, *Proc. Natl. Acad. Sci. USA* 96:6120-6106, 1999.

Amino acid alterations can be designed to maintain ligand binding or receptor activity taking into account the structure and property of different amino acids. Depending upon an amino acid side chain ("R" group), amino acids will have different properties such as size, polarity, the ability to hydrogen bond, and hydrophobicity. The effect of different amino acid side chains on properties of an amino acid are well known in the art. (See, for example, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-2001, Appendix 1C.)

In exchanging amino acids to maintain activity, the replacement amino acid should have similar properties. For example, substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

In exchanging amino acids to diminish an agonist interaction, the replacement amino acid should have a side chain not able to make the same type of interaction as the amino acid being replaced. For example neutral and hydrophobic amino acids (alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine), are good candidates for diminishing a hydrogen bond interaction. Proline because of its more restricted set of main chain conformations is generally not preferred.

In different embodiments the mutated ligand binding domain, which may be part of a transcription factor, is structurally similar to the ligand binding domain present in SEQ ID NOs: 1, 2, or 3. A structurally similar sequence is at least about 90% identical or similar to a

reference sequence. In different embodiments, a structural similar sequence is at least about 95% identical or similar, or at least about 99% identical or similar, to a reference sequence; or differs from the reference sequence by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid alterations.

Percent identity can be calculated by determining the minimum number of amino acid alterations to an amino acid sequence required to arrive at a reference sequence divided by the number of amino acids in the reference sequence multiplying by 100, then subtracting 100 by the obtained number. Amino acid alterations can be any combination of additions, deletions, or substitutions. The amino acid sequence compared to a reference sequence can be part of a larger sequence.

Sequence similarity for polypeptides can be determined by BLAST. (Altschul, *et al.*, 1997. *Nucleic Acids Res.* 25, 3389-3402, hereby incorporated by reference herein.) In one embodiment sequence similarity is determined using tBLASTn search program with the following parameters: MATRIX:BLOSUM62, PER RESIDUE GAP COST: 11, and Lambda ratio: 1.

In different embodiments, the transcription factor contains a mutated ligand binding domain described herein for PPAR α , PPAR δ , or PPAR γ . In preferred embodiments, the transcription factor consists of the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6. SEQ ID NO: 5 contains a Tyr473Ala alteration, while SEQ ID NO: 6 contains a Tyr473Phe alteration. SEQ ID NOs: 5 and 6 are as follows:

SEQ ID NO: 5:

MKLLSSIEQACDICRLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLTRAHLTEVES
 RLERLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQDNVNKDAVTDRLASVETDM
 PLTLRQHRISATSSSEESSNKGQRQLTVSPGIRMSHNAIRFGRMPQAEKEKLLAEISSDID
 QLNPEADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKI
 KFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVH
 EIIYTMLASLMNKDGVLISEGQGFMTREFLKSRLKPFQDFMEPKFEFAVKFNALELDDSD
 LAIFIAVILSGDRPGLLNPKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQI
 VTEHVQLLQVIKKTETDMSLHPLLQEIAKDLY

SEQ ID NO: 6:

MKLLSSIEQACDICRLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLTRAHLTEVES
 RLERLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQDNVNKDAVTDRLASVETDM
 PLTLRQHRISATSSSEESSNKGQRQLTVSPGIRMSHNAIRFGRMPQAEKEKLLAEISSDID

QLNPESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKI
 KFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVH
 EIITYTMLASLMNKDGVLISEGQGFMTREFLKSLRKPFMGDFMEPKFEFAVKFNALELDDSD
 LAIFIAVILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQI
 5 VTEHVQLLQVIKKTETDMSLHPLLQEIFKDLY

Polypeptide Production

Polypeptides can be produced using standard techniques including those involving chemical synthesis and those involving biochemical synthesis. Techniques for
 10 chemical synthesis of polypeptides are well known in the art. (See *e.g.*, Vincent, in *Peptide and Protein Drug Delivery*, New York, N.Y., Dekker, 1990.)

Biochemical synthesis techniques for polypeptides are also well known in the art. Examples of techniques for introducing nucleic acid into a cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, *Current Protocols in Molecular*
 15 *Biology*, John Wiley, 1987-1998, and Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded by different
 20 combinations of nucleotide triplets or "codons". Amino acids are encoded by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU

C=Cys=Cysteine: codons UGC, UGU

D=Asp=Aspartic acid: codons GAC, GAU

E=Glu=Glutamic acid: codons GAA, GAG

25 F=Phe=Phenylalanine: codons UUC, UUU

G=Gly=Glycine: codons GGA, GGC, GGG, GGU

H=His=Histidine: codons CAC, CAU

I=Ile=Isoleucine: codons AUA, AUC, AUU

K=Lys=Lysine: codons AAA, AAG

30 L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU

M=Met=Methionine: codon AUG

N=Asn=Asparagine: codons AAC, AAU

P=Pro=Proline: codons CCA, CCC, CCG, CCU

Q=Gln=Glutamine: codons CAA, CAG

35 R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

W=Trp=Tryptophan: codon UGG

5 Y=Tyr=Tyrosine: codons UAC, UAU

Nucleic acid encoding a mutated ligand binding domain can be obtained by producing a nucleic acid using chemical synthesis techniques or by mutating a previously synthesized nucleic acid. Mutating a previously synthesized nucleic acid is facilitated using techniques such as site directed mutagenesis which can be employed to alter a particular
10 nucleotide to obtain a desired codon.

Recombinant Expression

Polypeptides are preferably expressed by recombinant nucleic acid in a suitable host or expression system. Recombinant nucleic acid is nucleic acid that by virtue of its
15 sequence or form does not occur in nature. Possible forms for recombinant nucleic acid include isolation from nucleic acid found in a cell; or a polypeptide encoding region combined with other nucleic acid, which may be present in a host genome or outside of the host genome.

More preferably, expression is achieved in a host cell using an expression vector. An expression vector is a recombinant nucleic acid that includes a region encoding a polypeptide
20 along with regulatory elements for proper transcription and processing. The regulatory elements that may be present include those naturally associated with the polypeptide encoding region and exogenous regulatory elements not naturally associated with the polypeptide coding region.

Exogenous regulatory elements such as an exogenous promoter can be useful for expressing recombinant nucleic acid in a particular host. An exogenous promoter for a
25 polypeptide containing a mutated PPAR ligand binding domain is a promoter that is not naturally associated with PPAR encoding nucleic acid.

Generally, the regulatory elements that are present in an expression vector include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in
30 eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

To enhance expression in a particular host it may be useful to modify a particular
35 encoding sequence to take into account codon usage of the host. Codon usage of different

organisms are well known in the art. (See, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

Expression vectors may be introduced into host cells using standard techniques. Examples of such techniques include transformation, transfection, lipofection, protoplast fusion, and electroporation.

Nucleic acid encoding a polypeptide can be expressed in a cell without the use of an expression vector. For example, mRNA can be translated in various cell-free systems such as wheat germ extracts and reticulocyte extracts, as well as in cell based systems, such as frog oocytes. Introduction of mRNA into cell based systems can be achieved, for example, by microinjection.

PPAR assays can be performed using a host expressing a mutated ligand binding domain polypeptide, and can be performed using a mutated ligand binding domain polypeptide purified from a host or expression system. Preferably, assays are performed using a recombinant cell.

A recombinant cell encoding a mutated PPAR ligand binding domain polypeptide is a cell that is modified to contain nucleic acid encoding the polypeptide. The modification can be by different methods, such as introduction of an expression vector and mutation of the host genome.

PPAR Assays Formats

Polypeptides containing a mutated PPAR ligand binding domain can be employed to evaluate and select for partial agonists. A variety of different assay formats can be employed including ligand binding assays, assays measuring coactivator affinity, and assay measuring transcription factor activity. Examples of different assay formats include:

- 1) Measuring ligand binding using a scintillation proximity assay format (e.g., Elbrecht *et al.*, *The Journal of Biological Chemistry* 12:7913-7922, 1999);
- 2) Measuring nuclear receptor affinity for cofactors using fluorescence resonance energy transfer (e.g., Zhou *et al.*, *Molecular Endocrinology* 12:1594-1604, 1998); and
- 3) Measuring transcription factor activity (e.g., Example Section *infra.*, Lehman *et al.*, *The Journal of Biological Chemistry* 270:12953-12956, 1995, Schmidt *et al.*, *Molecular and Cellular Endocrinology* 155:51-60, 1999, Berger *et al.*, *The Journal of Biological Chemistry* 274:6718-6725, 1999.)

Full and partial agonists can be discriminated, for example, by running two simultaneous transactivation assays one involving the wild-type receptor (native or chimera) and the other involving the mutated receptor. Ligands having severely diminished activity in the

mutant assay versus wild-type are classified as full agonists. Ligands that exhibit the same activity or enhanced activity in the mutant assay versus wild-type can be classified as partial agonists.

5

EXAMPLES

Examples are provided below further illustrating different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

10 Example 1: Mutated Ligand Binding Domain Construction

Mutated PPAR γ ligand binding domain polypeptides were generated by site directed mutagenesis of encoding nucleic acid, followed by nucleic acid expression. The starting construct for mutagenesis was pcDNA3-hPPAR γ /GAL4. pcDNA3-hPPAR γ /GAL4 is a chimeric transcription factor containing a human hPPAR γ ligand binding domain and a yeast GAL4
15 transcription factor DNA binding domain.

pcDNA3-hPPAR γ /GAL4 was prepared by inserting the yeast GAL4 transcription factor DNA binding domain adjacent to the ligand binding domain of human PPAR γ within the mammalian expression vector pcDNA3.1(+). Construction was achieved using techniques described by Elbrecht *et al. J. Biol. Chem.* 274:7913-7922, 1999.

20 Starting with pcDNA3-hPPAR γ /GAL4, the Tyr473 residue of human PPAR γ was mutated to Ala or Phe by utilizing the Quikchange Site-Directed Mutagenesis Kit according to the protocol of the manufacturer (Stratagene, La Jolla, CA). The Tyr473Ala mutation was made using the forward oligonucleotide 5'-GCTCCTGCAGGAGATCGCCAAGGACTTGTACTAG-3' (SEQ ID NO: 9) and the reverse oligonucleotide 5'-

25 CTAGTACAAGTCCTTGGCGATCTCCTGCAGGAGC-3' (SEQ ID NO: 10). The Tyr473Phe mutation was made using the forward oligonucleotide 5'-GCTCCTGCAGGAGATCTTCAAGGACTTGTACTAG-3' (SEQ ID NO: 11) and the reverse oligonucleotide 5'-CTAGTACAAGTCCTTGAAGATCTCCTGCAG GAGC-3' (SEQ ID NO: 12).

30 The mutated constructs containing a PPAR γ ligand binding alteration in Tyr473 were designated pcDNA3-PPAR γ (473Ala)/GAL4, or pcDNA3-PPAR γ (473Phe)/GAL4. The nucleic acid sequence encoding the GAL4/PPAR γ (473 Ala) construct is provided by SEQ ID NO: 7. The nucleic acid sequence encoding the GAL4/PPAR γ (473 Phe) construct is provided by SEQ ID NO: 8.

35

Example 2: Transactivation Assay

A transactivation assay was performed to evaluate mutated PPAR PPAR γ ligand binding domains. The transcription assay employed the transcription factors described in Example 1 and a reporter plasmid. Expression of the reporter plasmid is induced by transcription factor activation.

The employed reporter plasmid for the GAL4 chimeric receptors (pUAS(5X)-tk-luc) contains five repeats of the GAL4 response element (UAS) upstream of a minimal thymidine kinase promoter that is adjacent to the luciferase gene. (Berger *et al.*, *J. Biol. Chem.* 274:6718-6725, 1999.) A control vector, pCMV-lacZ, contains the CMV promoter adjacent to the galactosidase Z gene. (Berger *et al.*, *J. Biol. Chem.* 274:6718-6725, 1999.)

Rosiglitazone ((+/-)-5-(4-(2-(methyl-2-pyridinylamino)ethoxy)phenyl)methyl)-2,4-thiazolidinedione) and Compound 1 were evaluated. Cell culture reagents were obtained from Gibco (Gaithersburg, MD). Unless otherwise noted, all other reagents were obtained from Sigma Chemicals (St. Louis, MO).

COS-1 cells were cultured and transactivation assays were performed using the expression vectors pcDNA3-PPAR γ /GAL4, pcDNA3-PPAR γ (473Ala)/GAL4, or pcDNA3-PPAR γ (473Phe)/GAL4 using techniques described by Berger *et al.*, *J. Biol. Chem.* 274:6718-6725, 1999. Briefly, cells were transfected with a transcription factor expression vector, pUAS(5X)-tk-luc reporter vector and pCMV-lacZ as an internal control for transactivation efficiency using Lipofectamine (Invitrogen, Carlsburg, CA). After a 48 hour exposure to compounds, cell lysates were produced, and luciferase and β -galactosidase activity in cell extracts was determined. (Berger *et al.*, *J. Biol. Chem.* 274:6718-6725, 1999.)

The PPAR γ full agonist rosiglitazone showed a dramatic diminution in potency in activating the PPAR γ Tyr473Ala mutant in comparison with wild-type PPAR γ (Figure 4). In contrast, the potency of Compound 1 in activating the PPAR γ Tyr473Ala mutant remained essentially unchanged while its efficacy (maximal response) was augmented in comparison with wild-type PPAR γ (Figure 4). The potency of rosiglitazone in activating the PPAR γ Tyr473Phe mutant was also greatly reduced in comparison with wild-type PPAR γ (Figure 5). The potency of Compound 1 in activating the PPAR γ Tyr473Phe remained similar while its efficacy was significantly augmented in comparison with wild-type PPAR γ (Figure 5).

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. A mutated peroxisome proliferator-activated receptor (PPAR) ligand binding domain polypeptide comprising the amino acid sequence of a mutated PPAR ligand binding domain, wherein said mutated PPAR ligand binding domain is
 - (a) bound by a partial PPAR agonist; and
 - (b) bound or activated by a full PPAR agonist to a lesser extent than the wild-type receptor.
2. The mutated PPAR ligand binding domain polypeptide of claim 1, wherein said mutated PPAR ligand binding domain selectively binds said partial agonist.
3. The mutated PPAR ligand binding domain polypeptide of claim 1, wherein said mutated PPAR ligand binding domain polypeptide is selectively activated by said partial agonist.
4. The mutated PPAR ligand binding domain polypeptide of claim 1, wherein said mutated ligand bind domain is either:
 - a mutated human PPAR α ligand binding domain, wherein a residue corresponding to tyrosine 464 is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine;
 - a mutated human PPAR δ ligand binding domain, wherein a residue corresponding to tyrosine 437 is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine, or
 - a mutated human PPAR γ ligand binding domain, wherein a residue corresponding to tyrosine 473 is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine.
5. The mutated PPAR ligand binding domain polypeptide of claim 1, where said polypeptide comprises the amino acid sequence of SEQ ID NO: 4:
QLNPESADLRALAKHLYDSYKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKI
KFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLKYG
VH
EIIYTMLASLMNKDGVLISEGQGFMTREFLKSRLKPFGDFMEPKFEFAVKFNALELDDSD
LAIFIAVILSGDRPGLLNVPKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQI
VTEHVQLLQVIKKTETDMSLHPLLQEIXKDLY

wherein X is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine.

5 6. The mutated PPAR ligand binding domain polypeptide of claim 5, wherein X is phenylalanine or alanine.

7. A ligand-activated transcription factor comprising the mutated PPAR ligand binding domain of claim 1 and a DNA binding domain.

10 8. The ligand-activated transcription factor of claim 7, wherein said transcription factor can be selectively activated by partial agonist binding.

9. The ligand-activated transcription factor of claim 8, wherein said mutated ligand bind domain is either:

15 a mutated human PPAR α ligand binding domain, wherein a residue corresponding to tyrosine 464 is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine;

20 a mutated human PPAR δ ligand binding domain, wherein a residue corresponding to tyrosine 437 is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine, or

 a mutated human PPAR γ ligand binding domain, wherein a residue corresponding to tyrosine 473 is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine.

25 10. The ligand-activated transcription factor of claim 7, where said mutated ligand binding domain consists of the amino acid sequence of SEQ ID NO: 4:
 QLNPEADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVTYDMNSLMMGEDKI
 KFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVH
 EIIYTMLASLMNKDGVLISEGQGFMTRFLKSLRKPFQDFMEPKFEFAVKFNALELDDSD
 30 LAIFIAVILSGDRPGLLNVPKPIEDIQDNLLQALELQLKLNHPSSQLFAKLLQKMTDLRQI
 VTEHVQLLQVIKKTETDMSLHPLLQEIXKDLY
 wherein X is selected from the group consisting of: alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, methionine, histidine, asparagine, and glutamine.

11. The ligand-activated transcription factor of claim 10, wherein X is phenylalanine or alanine.

12. The ligand-activated transcription factor of claim 11, wherein said transcription factor is a chimeric receptor.

13. The ligand-activated transcription factor of claim 12, wherein said transcription factor consists of the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 6.

14. A method of making a mutated PPAR ligand binding domain polypeptide comprising the step of mutating a PPAR ligand binding domain such that an amino acid present in a wild-type PPAR ligand binding domain that makes a direct interaction with a full agonist either makes no interaction, or a substantially different interaction, with said full agonist.

15. The method of claim 14, wherein said mutating produces said mutated PPAR ligand binding domain polypeptide such that said mutated PPAR ligand binding is selectively bound or activated by a partial PPAR agonist.

16. The method of claim 15, wherein said mutating comprises changing an amino acid that makes a direct interaction with a full agonist into an amino acid that either makes no interaction, or a substantially different interaction, with said full agonist.

17. The method of claim 16, wherein said PPAR ligand binding domain that is mutated comprises SEQ ID NO: 3:

QLNPESADLRALAKHLYDSYIKSFPLTKAKARAILTGKTTDKSPFVIYDMNSLMMGEDKI
KFKHITPLQEQSKEVAIRIFQGCQFRSVEAVQEITEYAKSIPGFVNLDLNDQVTLLKYGVH
EIIYTMLASLMNKDGVLISEGQGFMTRFLKSLRKPFGDFMEPKFEFAVKFNALELDDSD
LAIFIAVILSGDRPGLLNVKPIEDIQDNLLQALELQLKLNHPESSQLFAKLLQKMTDLRQI
VTEHVQLLQVIKKTETDMSLHPLLQEIYKDLY.

18. A nucleic acid comprising a nucleotide sequence encoding the polypeptide of any one of claims 1-6 or the transcription factor of any one claims 7-13.

19. The nucleic acid of claim 18, wherein said nucleotide sequence is transcriptionally coupled to an exogenous promoter.

20. The nucleic acid of claim 19, wherein said nucleic acid is an expression vector.

5 21. A recombinant cell comprising the nucleic acid of claim 20, wherein said nucleic acid is expressed in said cell.

22. A method of assaying for a partial PPAR agonist comprising the step of measuring the ability of a test compound to bind to or activate the polypeptide of any one of
10 claims 1-6 or the transcription factor of any one of claims 7-13.

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10	20	30	40	50	60
MVDTESPLCP	LSPLEAGDLE	SPLSEEFLOE	MGNIQEISQS	IGEDSSGSFG	FTEYQYLGSC
70	80	90	100	110	120
PGSDGSPVITD	TLSPASSPSS	VTYPVVPGSV	DESPSGALNI	ECRICGDKAS	GYHYGVHACE
130	140	150	160	170	180
GCKGFFRRTI	RLKLVYDKCD	RSCKIQKKNR	NKCQYCRFHK	CLSVGMSHNA	IRFGRMPRSE
190	200	210	220	230	240
KAKLKAEILT	CEHDIEDSET	ADLKSLAKRI	YEAYLKNFNM	NKVKARVILS	GKASNNPPFV
250	260	270	280	290	300
IHDMETLCMA	EKTLVAKLVA	NGIQNKEAEV	RIFHCCQCTS	VETVTELTEF	AKAIPGFANL
310	320	330	340	350	360
DLNDQVTLLK	YGVYEAI FAM	LSSVMNKDGM	LVAYGNGFIT	REFLKSLRKP	FCDIMEPKFD
370	380	390	400	410	420
FAMKFNALEL	DDSDISLFVA	AIICCGDRPG	LLNVGHIEKM	QEGIVHVLRL	HLQSNHPDDI
430	440	450	460		
FLFPKLLQKM	ADLRQLVTEH	AQLVQIIKKT	ESDAALHPLL	QEIYRDMY	

FIG. 1

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10	20	30	40	50	60
MEQPQEEAPE	VREEEEKEEV	AEAEGAPELN	GGPQHALPSS	SYTDLSRSSS	PPSLLDQLQM
70	80	90	100	110	120
GCDGASCGSL	NMECRVCGDK	ASGFHYGVHA	CEGCKGFFRR	TIRMKLEYEK	CERSCKIQKK
130	140	150	160	170	180
NRNKCQYCRF	QKCLALGMSH	NAIRFGRMPE	AEKRKLVAGL	TANEGSQYNP	QVADLKAFSK
190	200	210	220	230	240
HIYNAYLKNF	NMTKKKARSI	LTGKASHTAP	FVIHDIETLW	QAEKGLVWKQ	LVNGLPPYKE
250	260	270	280	290	300
ISVHVFYRCQ	CTTVETVREL	TEFAKSIPSF	SSLFLNDQVT	LLKYGVHEAI	FAMLASIVNK
310	320	330	340	350	360
DGLLVANGSG	FVTREFLRSL	RKPFSDIIEP	KFEFAVKFNA	LELDDSDLAL	FIAAIIILCGD
370	380	390	400	410	420
RPGLMNVPRV	EAIQDTILRA	LEFHLQANHP	DAQYLFPKLL	QKMADLRQLV	TEHAQMMORI
430	440				
KKTETETSLH	PLLQEIKDM	Y			

FIG. 2

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10	20	30	40	50	60
MTMVDTEMPF	WPTNFGISSV	DLSVMEDHSH	SFDIKPFTTV	DFSSISTPHY	EDIPFTRTDP
70	80	90	100	110	120
VVADYKYDLK	LQEYQSAIKV	EPASPPYYSE	KTQLYNKPHE	EPSNSLMAIE	CRVCGDKASG
130	140	150	160	170	180
FHYGVHACEG	CKGFFRRTIR	LKLIYDRCDL	NCRIHKKSRN	KCQYCRFQKC	LAVGMSHNAI
190	200	210	220	230	240
RFGRMPQAEK	EKLLAEISSD	IDQLNPESAD	LRALAKHLYD	SYIKSFPLTK	AKARAILTGK
250	260	270	280	290	300
TTDKSPFVIY	DMNSLMMGED	KIKFKHITPL	QEQSKEVAIR	IFQGCQFRSV	EAVQEITEYA
310	320	330	340	350	360
KSIPGFVNLD	LNDQVTLLKY	GVHEIIYTML	ASLMNKDGVL	ISEGQGFMTR	EFLKSLRKPF
370	380	390	400	410	420
GDFMEPKFEF	AVKFNALELD	DSDLAIPIAV	IILSGDRPGL	LNVKPIEDIQ	DNLLQALELQ
430	440	450	460	470	
LKLNHPESSQ	LFAKLLQKMT	DLRQIVTEHV	QLLQVIKKTE	TDMSLHPLLQ	EIYKDLY

FIG. 3

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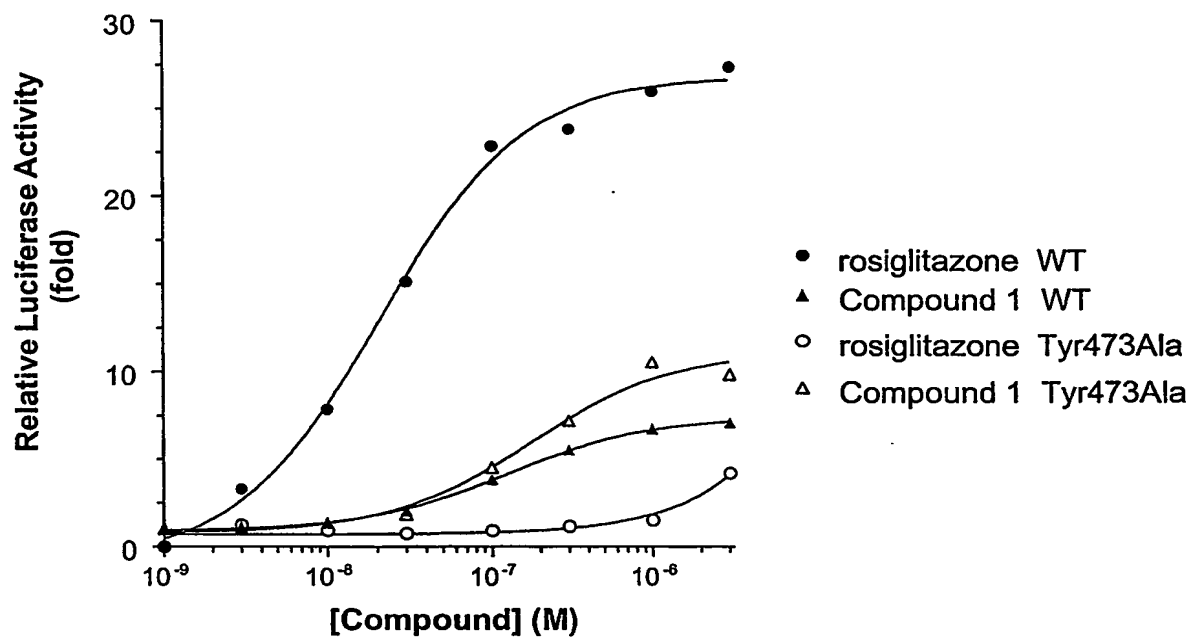


FIG. 4

5/5

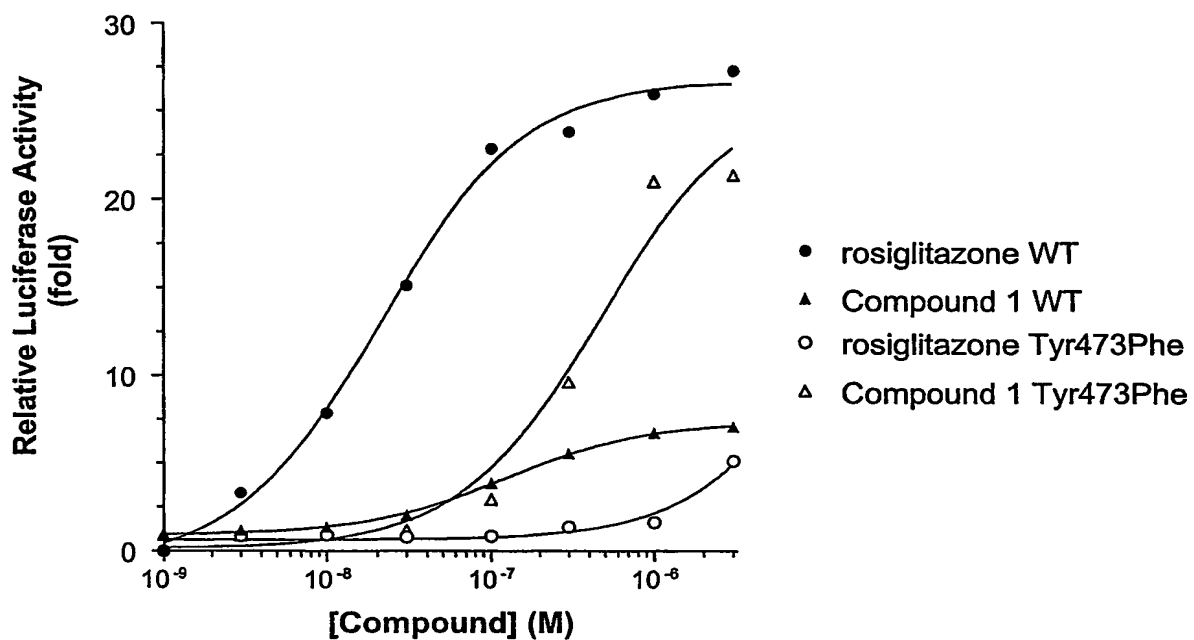


FIG. 5

SEQUENCE LISTING

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Phe Gly Phe Thr Glu Tyr Gln Tyr Leu Gly Ser Cys Pro Gly Ser Asp
 50          55          60
Gly Ser Val Ile Thr Asp Thr Leu Ser Pro Ala Ser Ser Pro Ser Ser
 65          70          75          80
Val Thr Tyr Pro Val Val Pro Gly Ser Val Asp Glu Ser Pro Ser Gly
          85          90          95
Ala Leu Asn Ile Glu Cys Arg Ile Cys Gly Asp Lys Ala Ser Gly Tyr
          100          105          110
His Tyr Gly Val His Ala Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg
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Thr Ile Arg Leu Lys Leu Val Tyr Asp Lys Cys Asp Arg Ser Cys Lys
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Ile Gln Lys Lys Asn Arg Asn Lys Cys Gln Tyr Cys Arg Phe His Lys
 145          150          155          160
Cys Leu Ser Val Gly Met Ser His Asn Ala Ile Arg Phe Gly Arg Met
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Pro Arg Ser Glu Lys Ala Lys Leu Lys Ala Glu Ile Leu Thr Cys Glu
          180          185          190
His Asp Ile Glu Asp Ser Glu Thr Ala Asp Leu Lys Ser Leu Ala Lys
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Arg Ile Tyr Glu Ala Tyr Leu Lys Asn Phe Asn Met Asn Lys Val Lys
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          245          250          255

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 325 330 335
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 His Tyr Glu Asp Ile Pro Phe Thr Arg Thr Asp Pro Val Val Ala Asp
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 65 70 75 80
 Glu Pro Ala Ser Pro Pro Tyr Tyr Ser Glu Lys Thr Gln Leu Tyr Asn
 85 90 95

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      115      120      125
Glu Gly Cys Lys Gly Phe Phe Arg Arg Thr Ile Arg Leu Lys Leu Ile
      130      135      140
Tyr Asp Arg Cys Asp Leu Asn Cys Arg Ile His Lys Lys Ser Arg Asn
      145      150      155      160
Lys Cys Gln Tyr Cys Arg Phe Gln Lys Cys Leu Ala Val Gly Met Ser
      165      170      175
His Asn Ala Ile Arg Phe Gly Arg Met Pro Gln Ala Glu Lys Glu Lys
      180      185      190
Leu Leu Ala Glu Ile Ser Ser Asp Ile Asp Gln Leu Asn Pro Glu Ser
      195      200      205
Ala Asp Leu Arg Ala Leu Ala Lys His Leu Tyr Asp Ser Tyr Ile Lys
      210      215      220
Ser Phe Pro Leu Thr Lys Ala Lys Ala Arg Ala Ile Leu Thr Gly Lys
      225      230      235      240
Thr Thr Asp Lys Ser Pro Phe Val Ile Tyr Asp Met Asn Ser Leu Met
      245      250      255
Met Gly Glu Asp Lys Ile Lys Phe Lys His Ile Thr Pro Leu Gln Glu
      260      265      270
Gln Ser Lys Glu Val Ala Ile Arg Ile Phe Gln Gly Cys Gln Phe Arg
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Ser Val Glu Ala Val Gln Glu Ile Thr Glu Tyr Ala Lys Ser Ile Pro
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Gly Phe Val Asn Leu Asp Leu Asn Asp Gln Val Thr Leu Leu Lys Tyr
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Leu Lys Ser Leu Arg Lys Pro Phe Gly Asp Phe Met Glu Pro Lys Phe
      355      360      365
Glu Phe Ala Val Lys Phe Asn Ala Leu Glu Leu Asp Asp Ser Asp Leu
      370      375      380
Ala Ile Phe Ile Ala Val Ile Ile Leu Ser Gly Asp Arg Pro Gly Leu
      385      390      395      400
Leu Asn Val Lys Pro Ile Glu Asp Ile Gln Asp Asn Leu Leu Gln Ala
      405      410      415
Leu Glu Leu Gln Leu Lys Leu Asn His Pro Glu Ser Ser Gln Leu Phe
      420      425      430
Ala Lys Leu Leu Gln Lys Met Thr Asp Leu Arg Gln Ile Val Thr Glu
      435      440      445
His Val Gln Leu Leu Gln Val Ile Lys Lys Thr Glu Thr Asp Met Ser
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Leu His Pro Leu Leu Gln Glu Ile Tyr Lys Asp Leu Tyr
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<211> 275

<212> PRT

<213> Artificial Sequence

<220>

<223> mutated PPAR ligand binding domain

<221> VARIANT

<222> 271

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proline, tryptophan, phenylalanine, methionine,
histidine, asparagine, or glutamine

<400> 4

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Tyr	Asp	Ser	Tyr	Ile	Lys	Ser	Phe	Pro	Leu	Thr	Lys	Ala	Lys	Ala	Arg
			20					25					30		
Ala	Ile	Leu	Thr	Gly	Lys	Thr	Thr	Asp	Lys	Ser	Pro	Phe	Val	Ile	Tyr
		35					40					45			
Asp	Met	Asn	Ser	Leu	Met	Met	Gly	Glu	Asp	Lys	Ile	Lys	Phe	Lys	His
	50					55					60				
Ile	Thr	Pro	Leu	Gln	Glu	Gln	Ser	Lys	Glu	Val	Ala	Ile	Arg	Ile	Phe
65					70					75					80
Gln	Gly	Cys	Gln	Phe	Arg	Ser	Val	Glu	Ala	Val	Gln	Glu	Ile	Thr	Glu
				85					90					95	
Tyr	Ala	Lys	Ser	Ile	Pro	Gly	Phe	Val	Asn	Leu	Asp	Leu	Asn	Asp	Gln
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Val	Thr	Leu	Leu	Lys	Tyr	Gly	Val	His	Glu	Ile	Ile	Tyr	Thr	Met	Leu
		115					120					125			
Ala	Ser	Leu	Met	Asn	Lys	Asp	Gly	Val	Leu	Ile	Ser	Glu	Gly	Gln	Gly
		130				135					140				
Phe	Met	Thr	Arg	Glu	Phe	Leu	Lys	Ser	Leu	Arg	Lys	Pro	Phe	Gly	Asp
145					150					155					160
Phe	Met	Glu	Pro	Lys	Phe	Glu	Phe	Ala	Val	Lys	Phe	Asn	Ala	Leu	Glu
				165				170						175	
Leu	Asp	Asp	Ser	Asp	Leu	Ala	Ile	Phe	Ile	Ala	Val	Ile	Ile	Leu	Ser
			180					185					190		
Gly	Asp	Arg	Pro	Gly	Leu	Leu	Asn	Val	Lys	Pro	Ile	Glu	Asp	Ile	Gln
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Asp	Asn	Leu	Leu	Gln	Ala	Leu	Glu	Leu	Gln	Leu	Lys	Leu	Asn	His	Pro
		210				215					220				
Glu	Ser	Ser	Gln	Leu	Phe	Ala	Lys	Leu	Leu	Gln	Lys	Met	Thr	Asp	Leu
225					230					235					240
Arg	Gln	Ile	Val	Thr	Glu	His	Val	Gln	Leu	Leu	Gln	Val	Ile	Lys	Lys
				245					250					255	
Thr	Glu	Thr	Asp	Met	Ser	Leu	His	Pro	Leu	Leu	Gln	Glu	Ile	Xaa	Lys
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Asp	Leu	Tyr													
		275													

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<211> 454

<212> PRT

<213> Artificial Sequence

<220>

<223> transcription factor containing a mutated PPAR
ligand binding domain

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 Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
 35 40 45
 Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
 50 55 60
 Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
 65 70 75 80
 Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
 85 90 95
 Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
 100 105 110
 Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
 115 120 125
 Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
 130 135 140
 Thr Val Ser Pro Gly Ile Arg Met Ser His Asn Ala Ile Arg Phe Gly
 145 150 155 160
 Arg Met Pro Gln Ala Glu Lys Glu Lys Leu Leu Ala Glu Ile Ser Ser
 165 170 175
 Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp Leu Arg Ala Leu Ala
 180 185 190
 Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu Thr Lys Ala
 195 200 205
 Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys Ser Pro Phe
 210 215 220
 Val Ile Tyr Asp Met Asn Ser Leu Met Met Gly Glu Asp Lys Ile Lys
 225 230 235 240
 Phe Lys His Ile Thr Pro Leu Gln Glu Gln Ser Lys Glu Val Ala Ile
 245 250 255
 Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser Val Glu Ala Val Gln Glu
 260 265 270
 Ile Thr Glu Tyr Ala Lys Ser Ile Pro Gly Phe Val Asn Leu Asp Leu
 275 280 285
 Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu Ile Ile Tyr
 290 295 300
 Thr Met Leu Ala Ser Leu Met Asn Lys Asp Gly Val Leu Ile Ser Glu
 305 310 315 320
 Gly Gln Gly Phe Met Thr Arg Glu Phe Leu Lys Ser Leu Arg Lys Pro
 325 330 335
 Phe Gly Asp Phe Met Glu Pro Lys Phe Glu Phe Ala Val Lys Phe Asn
 340 345 350
 Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Ile Phe Ile Ala Val Ile
 355 360 365
 Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu Asn Val Lys Pro Ile Glu
 370 375 380
 Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu Glu Leu Gln Leu Lys Leu
 385 390 395 400
 Asn His Pro Glu Ser Ser Gln Leu Phe Ala Lys Leu Leu Gln Lys Met
 405 410 415
 Thr Asp Leu Arg Gln Ile Val Thr Glu His Val Gln Leu Leu Gln Val
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Ile Lys Lys Thr Glu Thr Asp Met Ser Leu His Pro Leu Leu Gln Glu
 435 440 445

Ile Ala Lys Asp Leu Tyr
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<211> 454

<212> PRT

<213> Artificial Sequence

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 35 40 45
 Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
 50 55 60
 Glu Gln Leu Phe Leu Ile Phe Pro Arg Glu Leu Asp Met Ile
 65 70 75 80
 Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
 85 90 95
 Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
 100 105 110
 Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
 115 120 125
 Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
 130 135 140
 Thr Val Ser Pro Gly Ile Arg Met Ser His Asn Ala Ile Arg Phe Gly
 145 150 155 160
 Arg Met Pro Gln Ala Glu Lys Glu Lys Leu Leu Ala Glu Ile Ser Ser
 165 170 175
 Asp Ile Asp Gln Leu Asn Pro Glu Ser Ala Asp Leu Arg Ala Leu Ala
 180 185 190
 Lys His Leu Tyr Asp Ser Tyr Ile Lys Ser Phe Pro Leu Thr Lys Ala
 195 200 205
 Lys Ala Arg Ala Ile Leu Thr Gly Lys Thr Thr Asp Lys Ser Pro Phe
 210 215 220
 Val Ile Tyr Asp Met Asn Ser Leu Met Met Gly Glu Asp Lys Ile Lys
 225 230 235 240
 Phe Lys His Ile Thr Pro Leu Gln Glu Gln Ser Lys Glu Val Ala Ile
 245 250 255
 Arg Ile Phe Gln Gly Cys Gln Phe Arg Ser Val Glu Ala Val Gln Glu
 260 265 270
 Ile Thr Glu Tyr Ala Lys Ser Ile Pro Gly Phe Val Asn Leu Asp Leu
 275 280 285
 Asn Asp Gln Val Thr Leu Leu Lys Tyr Gly Val His Glu Ile Ile Tyr
 290 295 300
 Thr Met Leu Ala Ser Leu Met Asn Lys Asp Gly Val Leu Ile Ser Glu
 305 310 315 320

Gly Gln Gly Phe Met Thr Arg Glu Phe Leu Lys Ser Leu Arg Lys Pro
 325 330 335
 Phe Gly Asp Phe Met Glu Pro Lys Phe Glu Phe Ala Val Lys Phe Asn
 340 345 350
 Ala Leu Glu Leu Asp Asp Ser Asp Leu Ala Ile Phe Ile Ala Val Ile
 355 360 365
 Ile Leu Ser Gly Asp Arg Pro Gly Leu Leu Asn Val Lys Pro Ile Glu
 370 375 380
 Asp Ile Gln Asp Asn Leu Leu Gln Ala Leu Glu Leu Gln Leu Lys Leu
 385 390 395 400
 Asn His Pro Glu Ser Ser Gln Leu Phe Ala Lys Leu Leu Gln Lys Met
 405 410 415
 Thr Asp Leu Arg Gln Ile Val Thr Glu His Val Gln Leu Leu Gln Val
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<223> nucleic acid sequence encoding GAL4/PPARγ (473
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 tctcccaaaa ccaaaaggct tccgctgact agggcacatc tgacagaagt ggaatcaagg 180
 ctagaaagac tggaacagct atttctactg atttttcctc gagaagacct tgacatgatt 240
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 aatgtgaata aagatgccgt cacagataga ttggccttcag tggagactga tatgcctcta 360
 acattgagac agcatagaat aagtgcgaca tcatcatcgg aagagagtag taacaaagg 420
 caaagacagt tgactgtatc gccggggatc cggatgtctc ataatgccat caggtttggg 480
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 aagtccttcc cgctgaccaa agcaaaggcg agggcgatct tgacaggaaa gacaacagac 660
 aaatcaccat tcgttatcta tgacatgaat tccttaatga tgggagaaga taaaatcaag 720
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<210> 8

<211> 1365

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<213> Artificial Sequence

<220>

<223> nucleic acid sequence encoding GAL4/PPAR γ (473 Phe)

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ctagaaagac tggaacagct atthtctactg atthtttctc gagaagacct tgacatgatt 240
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aatgtgaata aagatgccgt cacagataga ttggcttcag tggagactga tatgcctcta 360
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ggctgccagt ttcgctccgt ggaggctgtg caggagatca cagagtatgc caaaagcatt 840
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<400> 10

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<400> 11
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<400> 12
ctagtacaag tccttgaaga tctcctgcag gagg

34

34

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Organization
International Bureau



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(72) Inventors; and

(75) Inventors/Applicants (for US only): **MOSLEY, Ralph, T.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **MCKEEVER, Brian Michael** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US). **BERGER, Joel, P.** [US/US]; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

(74) Common Representative: **MERCK & CO. INC.**; 126 East Lincoln Avenue, Rahway, New Jersey 07065-0907 (US).

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(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR

(57) Abstract: The present invention features mutated forms of PPAR ligand binding domain polypeptides that: (1) bind a partial PPAR agonist; and (2) is bound or activated by a full PPAR agonist to a lesser extent than the wild-type receptor. The mutated ligand binding domain contains an amino acid sequence wherein one or more interactions that preferentially (preferably solely) occurs between a full PPAR agonist and the AF-2 domain of a wild-type PPAR are modified. Preferably, the mutated ligand binding domain is selectively bound or activated by a partial PPAR agonist.



WO 2004/067711 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/01221

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : C07K 1/00, 14/00, 16/00, 17/00; A61K 38/24, 38/27 US CL : 530/350, 399 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 530/350, 399 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WEST 2.0		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y --- A	US 6,266,622 B1 (SCANLAN et al) 24 July 2001 (24.07.2001), Figures 16-20 and 31, columns 1-26, 31, 32, 47 and 48.	1-12 and 14-22 ----- 13
Y --- A	CUNNINGHAM, B.C. et al. High-Resolution Epitope Mapping of hGH-Receptor Interactions by Alanine-Scanning Mutagenesis. Science. 2 June 1989, Vol. 244, pages 1081-1085.	1-12 and 14-22 ----- 13
Y --- A	US 6,294,559 B1 (SMITH) 25 September 2001 (25.09.2001), columns 1-8 and attached Sequence Comparisons A-D).	1-12 and 14-22 ----- 13
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
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"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 30 June 2005 (30.06.2005)	Date of mailing of the international search report 17 8 JUL 2005	
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Robert Landsman <i>[Signature]</i> Telephone No. 571-272-1600 <i>[Signature]</i>	

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